Osmotic Equilibration of Marine Algae

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In an osmotically responsive biological system, the product of the water contents of the system when equilibrated with an external solution of tonicity T_o, and that tonicity, equal a constant. On the basis of an analogy to the ideal gas laws this formulation has been employed to quantify the osmotic relationships between cells and their external media (Harris, 1956; LeFevre, 1964). It is assumed that during the equilibration of the system in question only water moves across the biological membranes separating the external media from the cellular water and that there is a negligible movement of solute. At osmotic equilibrium the tonicity of the external media is assumed to be equal to the tonicity of the cellular water.

Gravimetrically this relationship between the water contents of the equilibrated biological system and the tonicity of the external media may be expressed,

$$(W_o/D)\ T = k$$

where W_o/D are the grams of water per gram dry weight in the biological system, T is the tonicity of the external media with which it is equilibrated, and k is a constant. The total wet weight divided by the dry weight of the biological system may be expressed,

$$W/D = (W_o + W_n + D)\ /D$$

where W/D is the wet weight divided by the dry weight, W_o the grams of osmotically responsive water, W_n the grams of water that do not respond osmotically, and D the dry weight (gm) of the biological material. Rearranging the second expression,

$$W_o/D = W/D - (W_n + D)\ /D$$

By substitution into the first expression and rearrangement,

$$W/D = k/T + (W_n + D)\ /D$$

Thus the relationship between W/D and 1/T should be a straight line, with an intercept equal to $(W_n + D)\ /D$ and a slope k, related to W_o/D (Tosteson, 1964).

In plant cells the presence of the cell wall permits the development of a pressure (turgor pressure) on the internal solution that in effect increases the "activity" of the water in the vacuole and

protoplasm of this cell type. Thus at osmotic equilibrium with an external solution, the "apparent" tonicity of the cellular water equals the tonicity of the external medium. However the true tonicity of the cellular water T_c is slightly greater than the tonicity of the external medium with which it "equilibrated" and this difference is balanced by the effect of the turgor pressure (Sutcliffe, 1968). Thus,

$$T_c = T_e - (\triangle T)$$

where $(\triangle T)$ is the effective reduction in the true tonicity of the cellular water caused by the existence of turgor pressure. In other terms the "activity" of the water in the cell at osmotic equilibrium is slightly less than that of the water in the external media. This effect is cancelled by the presence of the turgor pressure which effectively increases the "activity" of the cellular water. In the work to be reported here the symbol T connotes the tonicity of the external solution with which the cells equilibrate, and equals the "effective" tonicity of the cellular water, that is the true tonicity minus the effect of the turgor pressure.

The turgor pressure has been shown in a number of algae to remain constant over a fairly wide range of environmental tonicities. Recently it has been suggested that the turgor pressure in the walled plant cell is regulated through adjustments in their salt uptake rates (Gutknecht, 1968). While the evaluation of this component in the osmotically equilibrated algal system is of importance no attempt will be made in the work reported here to quantitate this factor.

Procedure

The algae employed in these studies were of three species, *Caulerpa racemosa*, *Ulva lactuca* and *Spyridia filamentosa*. Samples of each type of algae were collected in the area of the Marine Station at La Parguera and brought directly to the laboratory. These samples were then prepared for experimentation, washed free of extraneous matter and foreign algae with millipore filtered sea water (pore size 0.45 micron) and placed in a large volume of filtered sea water in an illuminated growth chamber at a temperature of 28-29 C. In the case of each type of algae, selected portions and/or entire plants were then placed in a series of sea water solutions of

varying tonicities. In the experiments performed with *Ulva* and *Spyridia* several individual plants were placed in each of the various experimental solutions. The preparation of the material to be used in the experiments with *Caulerpa* (var. *laetevirens*) involved the separation of the assimilator from its rhizome. In the area of the connection to its rhizome the assimilator was twisted and pinched. A period of from 5-10 minutes was allowed for the formation of new crosswalls, following which the assimilator was cut from the rhizome. These isolated assimilators were placed in a constant flow aquarium for 12 hours prior to experimentation. The assimilators employed were examined visually for damage prior to their use. The index of damage in this case was the appearance of discoloration.

The three to four experimental tonicities employed in each experiment generally varied from two-thirds that of normal sea water to 1 1/3. The hypotonic sea water solutions were prepared by the addition of distilled water to the sea water and the hypertonic solutions by the addition of NaCl. All solutions were filtered through millipore filters (pore size 0.45 micron) prior to their use. The algae were exposed to these altered tonicities for varying lengths of time (from 1-24 hours) in the illuminated growth chamber. At the conclusion of a given experiment (of a given duration) each of the samples that were exposed to each of the tonicities in question was weighed. Following the determination of the wet weight of the sample in each case, the algae were dried either in an oven (105-110 C) to a constant dry weight (2 hours) or using the Ohaus Drying Balance at temperatures of 110-111 C. The latter procedure was employed with the *Ulva* and *Spyridia* samples and the former was employed with the samples of *Caulerpa*. In the procedure utilizing the Ohaus Drying Balance, constant dry weights were obtained in general after 25-30 minutes. The dry weight of the samples of *Caulerpa* were determined on an analytical balance.

Thus in the case of each algal sample exposed to a set of different tonicities for a given length of time, the quantity W/D was determined. The solutions in which these samples were equilibrated were individually filtered through millipore filters (pore size 0.45 micron) following the experimental incubation time. The tonicities of those solutions were then determined with the Fiske Freezing Point Depression Osmometer. The volume of solution employed in

the incubation of the samples in question was kept large (150-250 ml) compared to the volume of plant material incubated. The tonicity of the external media with which the algae equilibrated in such a case essentially remained constant, suggesting the absence of or at least rendering osmotically insignificant any changes in the solute distribution within the system.

The equilibration of the algae in question was studied over a period of from 18-24 hours, illuminated and at the temperature cited above. For each set of tonicities at a given incubation time, the relationship between the quantities W/D and 1/T was determined. In each case the value of W/D at isotonicity ((W/D) iso) was determined. Isotonicity in the *Caulerpa* experiments was 1,043 milliosmols/kg water ($(1/T) \times 10^3 = 0.959$) and in the case of the *Ulva* and *Spyridia* experiments 1, 026 milliosmols/kg water ($(1/T \times 10^3 = 0.975$). The value (W/D) iso $- 1 = (W_o + W_n)/D$, the isotonic water content in grams water/gram dry weight of the algal sample in question. The intercept of the relation between W/D and 1/T at a value of $1/T = 0$, gives the value of $(W_n + D)/D$. The value of (W/D) iso $-$ intercept $= W_o/D$, the grams of water osmotically responsive in the plant tissue per gram dry weight of that tissue. The fraction of the total isotonic water content that is osmotically responsive can thus be calculated, $W_o/(W_o + W_n)$. These parameters, the total isotonic water content of the plant tissue and the fraction of that water osmotically responsive were followed as a function of equilibration time in the three types of algae employed in this study.

The survival of the algal samples employed in these studies was determined by employing duplicate sets of tissues in each incubation medium used in each experiment. One of these tissues was used to determine the equilibrated W/D and the other was employed to test the viability of the sample after the completion of the experimental incubation period. The *Caulerpa* samples used to assess survival were removed from their respective incubation medias and placed in a continuous flow sea water aquarium at ambient light and temperature conditions for seven days. Discoloration was used as an index of death in this case.

Samples of *Ulva* and *Spyridia* were tested for survival after being exposed to the experimental conditions outlined above by placing treated samples in small wire baskets which were then

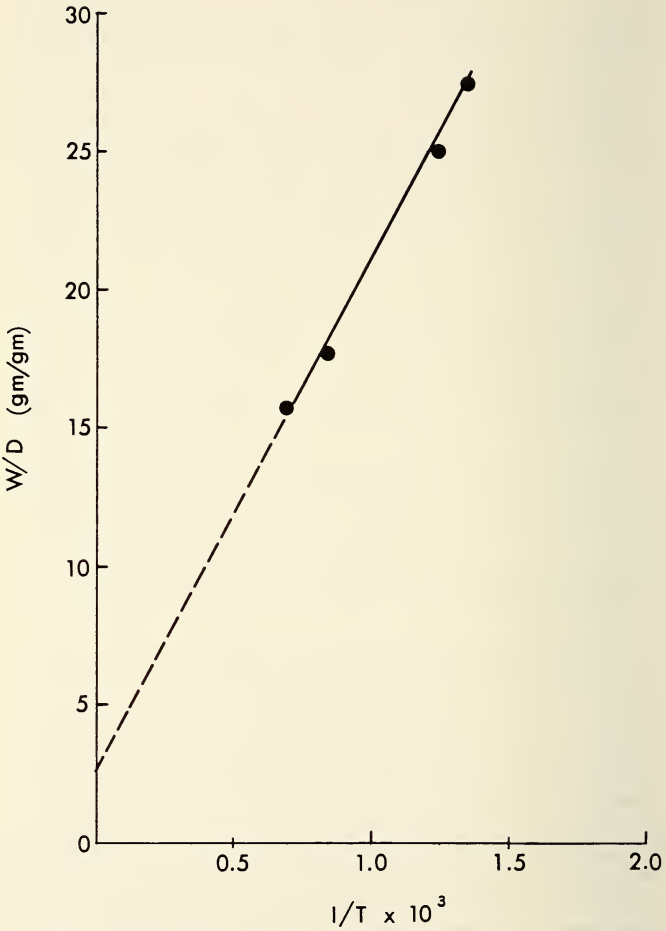

Fig. 1. Relationship between quantities W/D and 1/T for an equilibration experiment with *Caulerpa racemosa*.

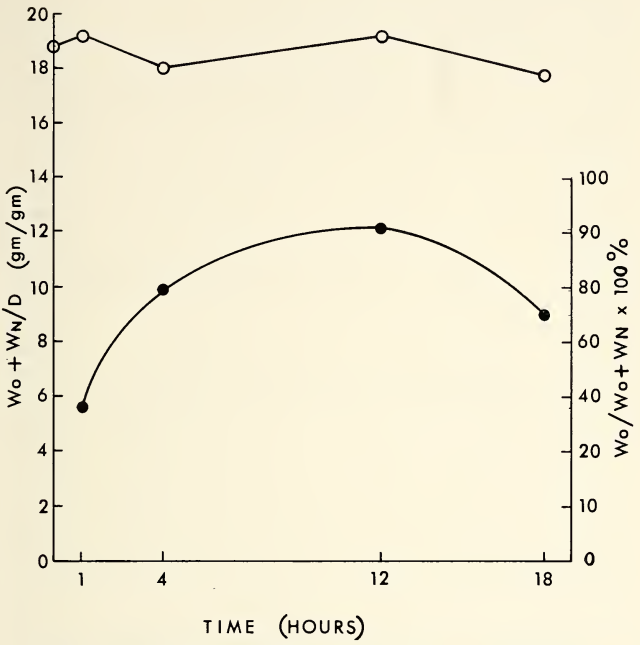

Fig. 2. Change in quantities W/D and 1/T as a function of time, in a series of six experiments with *Caulerpa racemosa*.

placed in the sea in the area of the marine station at La Parguera. Seven days later these samples were examined. Disintegration and discoloration were taken as an index of death.

Results

Caulerpa racemosa. The range of tonicities employed in the experiments reported here was from 1,349 milliosmols/kg water on the hypertonic side to a hypotonicity of 733 milliosmols/kg water. Figure 1 illustrates the relationship between the quantities W/D and 1/T for one of the equilibration experiments with *Caulerpa*. The demonstrated linearity in the relationship of W/D to 1/T was found

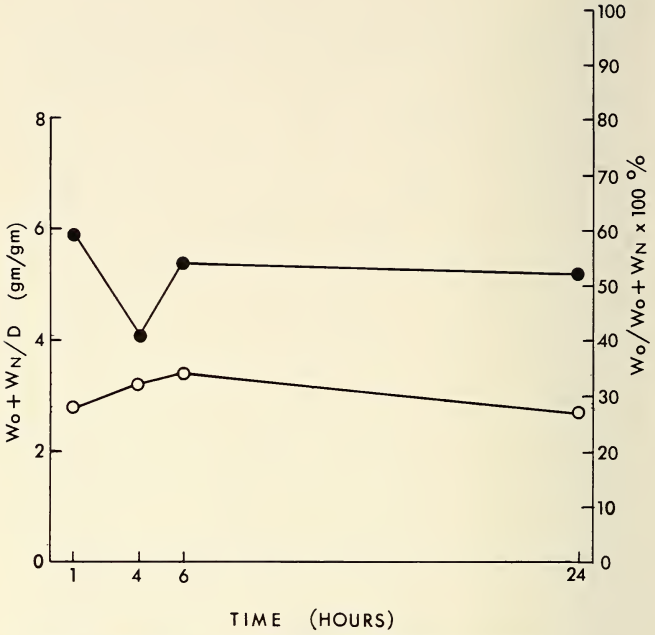

Fig. 3. Results of 10 equilibration experiments with *Ulva lactuca*.

to be true in all of the experiments with this alga. A similar linearity was found in the case of the experiments utilizing the algae *Ulva lactuca* and *Spyridia filamentosa*. On the basis of the linear regressions, computed by the method of least squares, the isotonic W/D and hence the isotonic water content ((W_o+W_n) /D) and the fraction of that water osmotically responsive (W_o/ (W_o+W_n)) were ascertained for each time of incubation (equilibration).

Figure 2 illustrates the change in these quantities as a function of time, in a series of six representative experiments with *Caulerpa*. The isotonic water contents of the assimilators of *Caulerpa* do not appear to change markedly with time of incubation. On the other hand the fraction of the water osmotically responsive appears to in-

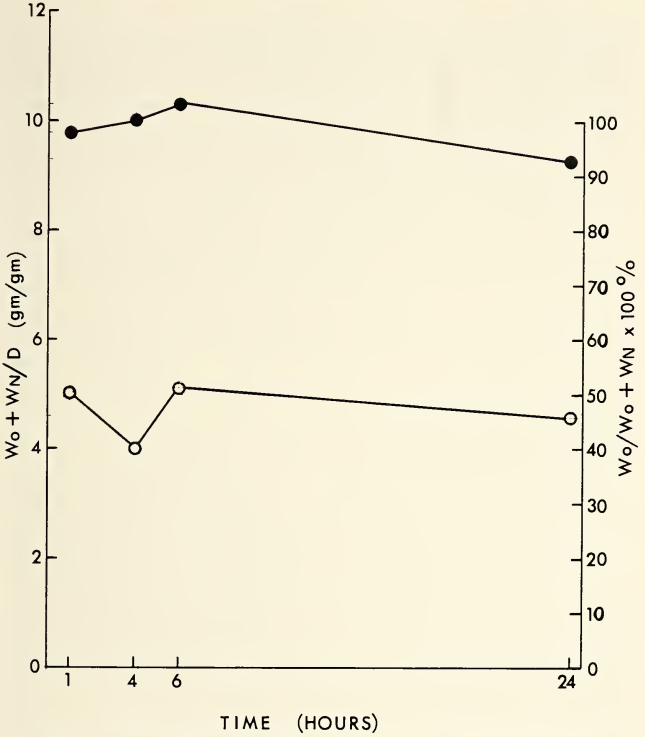

Fig. 4. Results of seven equilibration experiments with *Spyridia filamentosa.*

crease to a value as high as 91 per cent in 12 hours of equilibration. This fraction decreases however in experiments of 18 hours duration. Thus while the linearity of the relation between W/D and 1/T is found at each of the incubation times, suggesting an osmotic equilibration, the water contents of the tissue appear to continue to alter. The isotonic water contents appear to stay constant (avg. 18.971 gm of water/gram dry weight), however, the quantity of water osmotically responsive increases with time up to 12 hours. Following this, $W_o/ (W_o + W_n)$ decreases in value.

The results of the survival determinations in the case of this alga, indicate that all of the experimentally treated samples survived up through 12 hours of incubation. After 18 hours of incubation only samples kept in isotonic sea water solutions survived and all those samples placed in altered tonicities (hypertonic or hypotonic) were no longer viable. Thus even variations in tonicity of ±226 mosmols from isotonicity, were deliterious to the survival of the assimilators after 18 hours of equilibration.

Ulva lactura. The results of 10 representative equilibration experiments with this alga are given in Fig. 3. In this graph the average figures for the parameters defined previously are given as a function of time of equilibration. The average isotonic water content (±SD) of *Ulva* is 5.097 ±.769 gm H_2O/gm dry weight. This parameter and the average fraction of water osmotically responsive (average 30.75 ±2.75 per cent) appear to be independent of the time of equilibration up to 24 hours. All samples of *Ulva lactuca* equilibrated over periods up to 24 hours (Fig. 3) survived the experimental conditions described.

Spyridia filamentosa. Figure 4 gives the results of the equilibration of this alga over periods up to 24 hours. There were seven equilibration experiments carried out in this case. The average isotonic water content of *Spyridia* is 9.85 ± .425 gm H_2O/gm dry weight. The average fraction of this total water osmotically responsive was 46.82 ± 5.08 per cent. These parameters appear to be independent of the time of equilibration up to 24 hours. Evaluation of the survival of this alga revealed that the samples survived all experimental conditions and equilibration times.

Discussion

The isotonic water contents of the algae used in these experiments varied considerably, from *Caulerpa* (18.971 gm/gm) to *Ulva* (5.097) gm/gm). All samples of algae appeared to initially equilibrate for the relation of W/D *versus* 1/T was essentially linear in each case. However only in the cases of *Spyridia* and *Ulva* were the algae able to maintain themselves in their equilibrated state for periods of time up to 24 hours. The samples of *Caulerpa* did not maintain themselves at the experimental tonicities employed. Thus the slope of the relationship between W/D and 1/T in this case was not independent of time. That this change represents an alteration

in the system which ultimately results in an irreversible damage is supported by the fact that *Caulerpa* does not survive 18 hours of equilibration whereas both *Ulva* and *Spyridia* do. This difference might be attributed to the complexities encountered in the preparation of the *Caulerpa* assimilators for the experiments described. However these assimilators prepared in the fashion described, isolated from their rhizomes, do survive and resume growth when exposed to ambient conditions. Those samples exposed to isotonic sea water in the experiments reported here also survive. Thus the experimental procedures do not appear to account entirely for the response of this alga to altered tonicities, for if there had been fundamental damage incurred in preparing the assimilators it is doubtful that those samples incubated in isotonic media or sea water in ambient conditions, would have been able to maintain their isotonic water content or survive.

The *Caulerpa* tissues exposed to hypotonic conditions slowly continued to gain water whereas those exposed to hypertonic conditions slowly continued to lose water. Thus the slope of the relation between W/D and 1/T continues to change (becomes greater) in time. These losses and gains of water are not reflected in changes in the total isotonic water contents of the plant tissue in time, but rather are reflected in the water contents of the osmotically responsive compartment exposed to an altered tonicity. Thus the *Caulerpa* assimilators do not appear to be able to equilibrate in the strict sense of the word. Following 12 hours of incubation the osmotic compartment exposed to hypotonic conditions begins to *lose* water, whereas in hypertonic conditions it begins to *gain* water (the slope of W/D vs. 1/T decreases). Such a condition would result if the respective compartments were to lose and gain salt. This condition suggests a marked change in the permeability of the membranes involved. At the equilibration time of 12 hours, 91 per cent of the isotonic water contents of this material are in the osmotically responsive compartment. The presence of a large quantity of water within this compartment suggests a considerable strain on the plasticity of the membrane components involved. Thus it is possible that a severely damaged or broken membrane might result from the swollen (or shrunken) osmotic component of a cell unable to equilibrate in a given tonicity. The decrease in the slope of W/D vs. 1/T after 12 hours of equilibration in the *Caulerpa*

samples suggests a sharp change in the permeability of the membrane enclosing the osmotically responsive compartment.

The results with the samples of *Ulva* and *Spyridia* indicate the ability of these algae to equilibrate and maintain themselves in the experimental conditions employed. The size of the osmotically responsive compartment in terms of its water content appears to be larger in the case of *Spyridia.*

In all three of the algae utilized in these experiments, in *Caulerpa* initially and in *Ulva* and *Spyridia* at osmotic equilibrium, a rather large fraction of the total isotonic water content of the plant is found to be osmotically unresponsive (W_n/D). In the case of the equilibrating algae, *Ulva* and *Spyridia,* approximately 60-70 per cent of the total isotonic water content appears to be either associated with a tissue compartment that does not respond osmotically or represents water structurally immobilized. Tissue water in the former case can be viewed as being associated with tissue spaces not delimited by a living membrane whereas in the former case this water would be "bound" to intercellular structure in such a manner as to prevent its responding to an osmotic gradient. While the latter case seems unlikely, as a result of the quantity of water involved, determinations of the true "extracellular space" in the plant tissues employed in these experiments were not made. The definition of the nature of this high fraction of osmotically un-responsive water in *Ulva* and *Spyridia* bears further experimentation. Such work is now in progress in this laboratory.

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